

What is claimed is:

1. An EPOa-hSA fusion protein, wherein at least one amino acid residue of the EPOa moiety of the fusion protein is altered such that a site which serves as a site for glycosylation in EPO does not serve as a site for glycosylation in the EPOa.  
5
2. The EPOa-hSA fusion protein of claim 1, wherein said fusion protein has the formula:  
10 R1-L-R2; R2-L-R1; or R1-L-R2-L-R1,  
wherein R1 is an erythropoietin analog amino acid sequence; L is a peptide linker and R2 is human serum albumin amino acid sequence.
3. The EPOa-hSA fusion protein of claim 2, wherein R1 and R2 are covalently  
15 linked via said peptide linker.  
20
4. The EPOa-hSA fusion protein of claim 1, wherein an amino acid residue which serves as an attachment point for glycosylation has been deleted.  
25
5. The EPOa-hSA fusion protein of claim 1, wherein an amino acid residue of human EPO which serves as a site for glycosylation has been replaced with an amino acid residue which does not serve as a site for glycosylation.  
30
6. The EPOa-hSA fusion protein of claim 1, wherein said amino acid residue is selected from the group consisting of amino acid residues Asn24, Asn38, Asn83 and Ser126.  
35
7. The EPOa-hSA fusion protein of claim 1, wherein said glycosylation site is altered at amino acid residue Ser126 and at least one additional N-linked glycosylation site selected from the group consisting of Asn24, Asn38 and Asn83 is altered.  
40
8. The EPOa-hSA fusion protein of claim 1, wherein said glycosylation site provides for N-linked glycosylation and is altered by replacing an amino acid residue Asn with Gln.  
45

9. The EPOa-hSA fusion protein of claim 1, wherein said glycosylation site provides for O-linked glycosylation and is altered by replacing an amino acid residue Ser with Gln.

5

10. The EPOa-hSA fusion protein of claim 1, wherein one or more of amino acid residues 24, 38, or 83 has been altered.

11. The EPOa-hSA fusion protein of claim 10, wherein one or more of amino  
10 acid residues 24, 38, or 83 has been replaced with Gln.

12. The EPOa-hSA fusion protein of claim 1, wherein amino acid residue 126 has been altered.

15        13. The EPOa-hSA fusion protein of claim 12, wherein said amino acid residue  
126 has been replaced with Ala.

14. The EPOa-hSA fusion protein of claim 1, wherein each of amino acid residues 24, 38, 83 and 126 has been altered such that it does not serve as a glycosylation site.

15. The EPOa-hSA fusion protein of claim 14, wherein each of said amino acid residues 24, 28, 83 and 126 has been replaced with Gln, Gln, Gln, and Ala respectively.

25        16. The EPOa-hSA fusion protein of claim 3, wherein said peptide linker is 10 to  
30 amino acids in length.

17. The EPOa-hSA fusion protein of claim 16, wherein each of said amino acids in said peptide linker is selected from the group consisting of Gly, Ser, Asn, Thr and Ala.

30

18. The EPOa-hSA fusion protein of claim 3, wherein said peptide linker includes a sequence having the formula (Ser-Ser-Ser-Ser-Gly)<sub>y</sub> wherein y is less than or equal to 8.

19. The EPOa-hSA fusion protein of claim 3, wherein said peptide linker includes a sequence having the formula ((Ser-Ser-Ser-Ser-Gly)<sub>3</sub>-Ser-Pro).
20. The EPOa-hSA fusion protein of claim 1, wherein the EPOa is Gln24,  
5 Gln38, Gln83, Ala126 EPO.
21. The EPOa-hSA fusion protein of claim 1, wherein the fusion protein includes from left to right, an EPOa which includes amino acid residues Gln24, Gln38, Gln83 and Ala126, a peptide linker, and human serum albumin.  
10
22. The EPOa-hSA fusion protein of claim 21, wherein the EPOa is Gln24, Gln38, Gln83, Ala126 EPO.
23. The EPOa-hSA fusion protein of claim 1, wherein the fusion protein is from  
15 left to right, Gln24, Gln38, Gln83, Ala126 EPO, a peptide linker having the formula ((Ser-Gly-Gly-Gly-Gly)<sub>3</sub>-Ser-Pro), and human serum albumin.
24. The EPOa-hSA fusion protein of claim 1, wherein the EPOa-hSA fusion protein includes, from left to right, human serum albumin, a peptide linker, and an EPOa  
20 which includes amino acid residues Gln24, Gln38, Gln83 and Ala126.
25. The EPOa-hSA fusion protein of claim 24, wherein the EPOa is Gln24, Gln38, Gln83, Ala126 EPO.
26. The EPOa-hSA fusion protein of claim 1, wherein the fusion protein is from  
25 left to right, human serum albumin, a peptide linker having the formula ((Ser-Gly-Gly-Gly-Gly)<sub>3</sub>-Ser-Pro), and Gln24, Gln38, Gln83, Ala126 EPO.
27. An isolated nucleic acid comprising a nucleotide sequence which encodes an  
30 EPOa-hSA fusion protein, wherein at least one amino acid residue of the encoded EPOa-hSA which can serve as a glycosylation site in EPO is altered such that it does not serve as a glycosylation site in the EPOa.

28. An expression vector or a construct which comprises the nucleic acid of claim 27.

29. A cell which comprises the vector or construct of claim 28.

5

30. A method of making an EPOa-hSA fusion in a construct or a vector, comprising forming in a construct or vector a sequence in which a nucleic acid which comprises a nucleotide sequence encoding an EPOa is linked in frame to a nucleic acid which comprises a nucleotide sequence encoding human serum albumin.

10

31. A method for making an EPOa-hSA fusion protein comprising:  
supplying a cell which comprises a nucleic acid which encodes an EPOa-hSA fusion protein and  
expressing said EPOa-hSA fusion protein from said nucleic acid, thereby  
making said EPOa-hSA fusion protein.

32. The method of claim 31, wherein said cell is selected from a group consisting of a mammalian, yeast, plant, insect or a bacterial cell.

20

33. A method of making an EPOa-hSA fusion protein comprising:  
providing a transgenic organism which includes a transgene which directs the expression of EPOa-hSA fusion protein;  
allowing the transgene to be expressed; and  
recovering EPOa-hSA fusion protein.

25

34. The method of claim 33 wherein, the transgenic organism is a transgenic animal.

30

35. The method of claim 33 wherein, the transgenic organism is a transgenic dairy animal.

36. The method of claim 33 wherein, the EPOa-hSA fusion protein is made in a mammary gland of a transgenic mammal under the control of a milk specific promoter.

37. The method of claim 36 wherein, said promoter is a milk serum protein or casein promoter.

38. The method of claim 37 wherein, the transgenic mammal is a goat.

5

39. A method for providing a transgenic preparation which includes an EPOa-hSA fusion protein in the milk of a transgenic mammal comprising:

providing a transgenic mammal having an EPOa-hSA fusion protein protein-coding sequence operatively linked to a promoter sequence that results in the expression of  
10 the protein-coding sequence in mammary gland epithelial cells,

allowing the fusion protein to be expressed, and obtaining milk from the mammal, thereby providing the transgenic preparation.

40. A transgenic organism, which includes a transgene which encodes an EPOa-hSA fusion protein.

41. The method of claim 40 wherein, the transgenic organism is a transgenic animal.

20 42. The method of claim 40 wherein, the transgenic organism is a transgenic dairy animal.

43. The method of claim 40 wherein, the EPOa-hSA fusion protein is made in a mammary gland of a transgenic mammal under the control of a milk specific promoter.

25

44. The method of claim 43 wherein, said promoter is a milk serum protein or casein promoter.

30

45. The method of claim 44 wherein, the transgenic mammal is a goat or cow.

46. A pharmaceutical composition having a therapeutically effective amount of an EPOa-hSA fusion protein.

47. A method of treating a subject in need of erythropoietin comprising administering a therapeutically effective amount of an EPOa-hSA fusion protein to the subject.

5 48. The method of claim 47, wherein the method comprises administering a nucleic acid encoding an EPO-hSA fusion protein to the subject.

49. The method of claim 48, wherein the nucleic acid is administered in a cell.

10 50. The method of claim 49, wherein the cell is an autologous cell.

51. An erythropoietin analog, wherein four sites which serve as sites for glycosylation in erythropoietin are altered such that they do not serve as glycosylation sites.

150  
P>Gln83, Ala126 EPO.

52. The erythropoietin analog of claim 48 wherein the EPOa is Gln24, Gln38,

53. The transgenic organism of claim 40, wherein the organism is a rabbit.

20 54. The transgenic organism of claim 40, wherein the organism is a bird.

55. A method for making an EPOa-hSA fusion protein in a cultured cell comprising supplying a cell which includes a nucleic acid which encodes an EPOa-hSA fusion protein, and expressing the EPOa-hSA fusion protein from the nucleic acid, thereby 25 making the EPOa-hSA fusion protein.

SEARCHED INDEXED  
SERIALIZED FILED  
APR 20 1992